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13. ABSTRACT *(Maximum 200 Words)*

High circulating levels of insulin-like growth factor-1 (IGF-1) or low levels of 1,25(OH)$_2$ vitamin D (1,25(OH)$_2$D) are associated with increased risk of prostate cancer. This project examines whether specific dietary patterns are related to prostate cancer by influencing levels of IGF-1 and 1,25(OH)$_2$D; specifically whether high energy and protein intakes increase IGF-1 and high intakes of calcium, phosphorus, and animal protein decrease 1,25(OH)$_2$D levels. The relationships between dietary factors and circulating 1,25(OH)$_2$D were examined in 630 men using the Massachusetts Male Aging Study. None of the hypothesized nutrients correlated significantly with 1,25(OH)$_2$D levels, suggesting that dietary modulation of vitamin D may not be a critical mechanism linking nutritional factors to prostate cancer. We are currently evaluating IGF-1 levels in the Health Professional Follow-Up Study database. After examining the relation between the dietary factors and IGF-1 level, we will use these data to formulate a predicted IGF-1 score for men in the Health Professional Follow-Up Study based on their responses to a dietary questionnaire, and then examine the predicted impact of the combined effect of pertinent dietary factors in relation to prostate cancer risk Ultimately, our aim is to understand mechanisms underlying how specific dietary factors influence development of prostate cancer.

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ANNUAL SUMMARY

Introduction

The scientific rationale for this Idea Grant is to clarify modifiable, mainly nutritional, determinants of levels of insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), 1,25$(OH)_2$ vitamin D (1,25$(OH)_2$D), and 25(OH)vitamin D (25(OH)D). High levels of IGF-1 and low 1,25$(OH)_2$D have been shown to be related to risk of prostate cancer [1]. Some dietary factors that hypothetically impact on these serological factors, including total energy intake, calcium intake and protein intake, have been associated with prostate cancer risk. Since the initial proposal, additional studies supporting an association between calcium intake and prostate cancer risk [2], and between IGF-1 and prostate cancer risk [3], have been published. Interest in the areas of IGF and vitamin D and cancer have been increasing. Thus, a need to determine factors, particularly modifiable ones, that impact on levels of these hormones clearly exists.

Report

Vitamin D: The tasks related to vitamin D have been completed. Specifically, 630 specimens were retrieved, thawed, and aliquotted from Massachusetts Male Aging Study serum samples that have been stored in freezers in the laboratory of Dr. Christopher Longcope at the University of Massachusetts, Worcester. The samples were then shipped by overnight courier to Dr. Bruce Hollis at the Medical University of South Carolina. Because a higher than expected number of samples with insufficient volume were found, 630 rather than the projected 900 samples were sent to the laboratories. The reduced number of specimens is not expected to adversely effect the conduct of the study appreciably, particularly because of the high quality control of the laboratories (the coefficients-of-variation (CV%) were 5.4% for 25(OH)D, 5.3% for 1,25$(OH)_2$D). The better than anticipated CV%s in both laboratories helps offset the reduced number of analyzable specimens because the power to detect correlations is determined both by the sample size and by the accuracy of the laboratory assay.

We have met our goals of the statistical analysis of dietary factors (e.g., calcium, phosphorus, fructose, animal protein) for prostate cancer in predicting concentrations of 1,25$(OH)_2$D. The mean values for 1,25$(OH)_2$D and 25(OH)D were 31.9 pg/ml and 24.1 ng/ml, respectively. These are well within the expected range. None of the hypothesized factors showed correlations with 1,25$(OH)_2$D. The Pearson partial correlation coefficients between the nutrients and 1,25$(OH)_2$D (adjusting for total energy) were as follows: calcium, $r = 0.066$ ($P = 0.10$); phosphorus, $r = 0.01$ ($P = 0.80$); animal protein, $r = -0.035$ ($P = 0.38$); and fructose, $r = 0.004$ ($P = 0.91$). In addition, we explored very high intakes of calcium and also did not find a lower level of 1,25$(OH)_2$D: for men consuming less than 1500 mg of calcium, mean 1,25$(OH)_2$D was 31.8 pg/ml; for calcium intakes 1500-1999 mg, the mean 1,25$(OH)_2$D was 33.7 pg/ml; and for those consuming >2000 mg of calcium, the mean 1,25$(OH)_2$D was 33.1 pg/ml. None of the nutrients correlated significantly with the 1,25$(OH)_2$D/25(OH)D ratio.

Unfortunately, these results do not support our original hypothesis that $1,25(OH)_2D$ is an important mediator of risk of several dietary risk factors of prostate cancer. While for some factors (fructose and animal protein) the proposed link with $1,25(OH)_2D$ was more speculative, the lack of association with calcium and phosphorus was surprising, given that metabolic studies clearly show that these impact on $1,25(OH)_2D$ levels on a short-term basis. While measurement error in the diet questionnaire may have accounted for some attenuation of an association, we do not expect that the measurement error was so large as to have entirely missed an important association. One of the original rationale of our study was that using the same methodology, we observed a strong relationship between calcium intake and risk of advanced prostate cancer; if $1,25(OH)_2D$ was the mediator of this relationship, we would have expected to observe a strong correlation between calcium and $1,25(OH)_2D$. These results suggest that dietary manipulation of $1,25(OH)_2D$ may not be the most feasible approach to prevent prostate cancer. Because we did not identify significant determinants of $1,25(OH)_2D$, we cannot derive an empirical model of $1,25(OH)_2D$ to use predict prostate cancer in the Health Professionals Follow-Up Study as proposed in Task 5.

Although our initial hypotheses were not supported, we will continue to examine the impact of nutrition and the vitamin D axis in relation to prostate cancer risk because the evidence remains that these factors are important, though probably not in the manner we had initially hypothesized. Firstly, we will explore other potential mechanisms whereby calcium may impact on risk of prostate cancer, particularly the impact of high calcium intakes on bone metabolism, as metastatic prostate cancer usually grows in bones. Using this dataset, we will also conduct secondary analyses of dietary and other modifiable factors that may affect $1,25(OH)_2D$ levels (such as specific food items, including fish and milk); by examining only nutrients thus far, we may have missed some important associations for food groups. Finally, recent research [4, 5] suggests that prostate cells have their own hydroxylase that converts 25(OH)D to $1,25(OH)_2D$; if true, then levels of 25(OH)D, rather than $1,25(OH)_2D$ as initially hypothesized, may be most relevant to prostate cancer risk. Thus, we will examine determinants of 25(OH)D more closely in this data base.

Insulin-like Growth Factors (IGFs): The second major focus of this grant besides vitamin D was to examine predictors of IGFs. As we had reported previously to the Grants Officer, when the IGF assays were started, Dr. Pollak noted that the concentrations were abnormally low. We spent several months determining whether there was a problem with assays in his laboratory, or whether there was substantial degradation in the samples. We concluded that there was degradation in the samples, which rendered them essentially unusable for IGF assays. This degradation did not affect the vitamin D samples. Fortunately, Dr. Pollak noted this problem early and assays were done on only a limited number of samples.

To achieve our aims, we proposed that for the IGF analyses, we use instead archived plasma samples from the Health Professionals Follow-Up Study, instead of samples from the Massachusetts Male Aging Study as initially proposed. This is an adequate

replacement for the aims of this study. In the Health Professionals Follow-Up Study, we have dietary information (using the same dietary instrument as the Massachusetts Male Aging Study) in 1986, 1990, and 1994. 16,000 archived blood samples were collected 1993-1994. We have already sent some of these samples to the laboratory of Dr. Pollak and there is no indication of the degradation problem. The multiple dietary questionnaires are actually an advantage over the Massachusetts Male Aging Study, which only had one. The primary goal of the study is to examine which dietary factors predict levels of IGF-1 and IGFBP-3, which are risk factors for prostate cancer. When we had initially applied for this project, an advantage of the Massachusetts Male Aging Study population was that laboratory assays had already been done for this population for various additional hormonal factors, such as testosterone, which also is a risk factor for prostate cancer, and it was of potential interest to examine IGFs and other hormones simultaneously, though this was not a specific aim of the proposal. We have subsequently examined some of these hormones in the Health Professionals Follow-Up Study, so there is no apparent disadvantage in using the Health Professionals Follow-Up Study data for the specific aim regarding IGF. This change was previously approved by the Grants Officer, and we are currently in the process of identifying appropriate samples from this cohort and sending the samples to Dr. Pollak's laboratory for analysis.

A technical issue raised from the last annual report was that no mention was made of IGF-2 determinations as indicated in the SOW. The mention of IGF-2 in the SOW was a typographical error. We had and have no intention of measuring IGF-2. IGF-2 was not mentioned in our proposal, nor were any funds requested for the assay. The mention of IGF-2 was inadvertently included in the SOW.

Key Research Accomplishments

- We have established low coefficients of variation in plasma level determinations for IGF-1, IGFBP-3, $1,25(OH)_2D$ and 25(OH)D.

- We have completed determinations for vitamin D metabolites and have conducted the primary analyses correlating nutritional factors to $1,25(OH)_2D$ levels. None of the hypothesized factors showed correlations with $1,25(OH)_2D$. Based on these results, we conclude that the mechanisms whereby these nutritional factors impact on prostate cancer risk are unlikely to be primarily through the vitamin D axis.

- We have found significant deterioration of IGF-1 and IGFBP-3 in the Massachusetts Male Aging Study samples. Thus, we have identified an alternative archived source of samples (the Health Professionals Follow-Up Study) and laboratory assays are now proceeding.

Reportable Outcomes (at this time)

None.

Conclusions

The scientific rationale for this Idea Grant is to clarify modifiable, mainly nutritional, determinants of levels of IGF-1, IGFBP-3, $1,25(OH)_2$ vitamin D ($1,25(OH)_2D$), and 25(OH)vitamin D. High levels of IGF-1 and low $1,25(OH)_2D$ have been shown to be related to risk of prostate cancer. Some dietary factors that hypothetically impact on these serological factors, including total energy intake, calcium intake and protein intake, have been associated with prostate cancer risk. Thus, a need to determine factors, particularly modifiable ones that impact on levels of these hormones clearly exists. In the current study, none of the hypothesized factors showed correlations with $1,25(OH)_2D$ in 630 men. The Pearson partial correlation coefficients (adjusting for total energy) were as follows: calcium: $r = 0.066$ ($P = 0.10$); phosphorus: $r = 0.01$ ($P = 0.80$); animal protein: $r = -0.035$ ($P = 0.38$); and fructose: $r = 0.004$ ($P = 0.91$). These results do not support our original hypothesis that 1,25 vitamin D is an important mediator of risk of several dietary risk factors of prostate cancer. These results suggest that other mechanisms whereby these dietary factors impact on prostate cancer need to be examined. We are continuing our study of the impact of dietary factors on levels of insulin-like growth factors.

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